Original Research

Journal of Advanced Veterinary Research
(2026) Volume 16, Issue 2, 223-226

Detection of FimH gene of *Klebsiella pneumoniae* isolate from quail cloacal swab in Surabaya city market

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ARTICLE INFO

Received: 06 October 2025

Accepted: 30 October 2025

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Keywords:

Quail, *K. pneumoniae*, FimH, Biofilm, public health.

ABSTRACT

Quail is an alternative poultry besides chicken but is susceptible to various diseases, one of which is Klebsiella pneumonia infection. *K. pneumoniae* bacteria have 4 well-known virulence factors, namely fimbriae, capsules, lipopolysaccharides (LPS), and siderophores. Fimbriae are hair-like proteins that extend from the cell surface. In *K. pneumoniae*, Fimbriae promote attachment to non-biological surfaces. Fimbriae are divided into types 1 and 3 according to the most studied. FimA, the major fimbriae subunit, FimH, and the minor apical adhesion protein make up type 1 fimbriae. FimH contains mannose and encourages adherence to the host surface. A robust biofilm is formed when bacteria bind to the surface, which is facilitated by increased expression of the FimH gene. The purpose of this study was to detect the FimH gene in *K. pneumoniae* in the Surabaya City market. A total of 130 cloacal swab samples were taken from five markets in Surabaya (Turi market, Cemara Pabean market, Bratang market, Kupang market, and Benowo market) and then planted on Mac Conkey agar and Gram stained on colonies suspected to be *K. pneumoniae*. Colonies on MCA media showed a pink color and were mucoid. Gram staining showed Gram-negative bacteria, short rods, single or paired. Biochemical testing was carried out with TSIA and iMViC (SIM, MR, VP, and SCA). Isolation and identification showed that the percentage of *K. pneumoniae* was found at 10% (13/130). PCR testing detected the FimH gene at 85% (11/13). *K. pneumoniae* bacteria carrying the FimH gene increase the risk of biofilm formation which can reduce the effectiveness of antibiotics to antibiotic resistance, thus potentially disrupting public health.

Introduction

The global demand for protein sources is met in large part by the chicken business, which is regarded as an essential industry (Khairullah et al., 2024; Wibisono et al., 2020). Nowadays, quail are acknowledged as a significant and promising alternative species that have numerous benefits over other poultry species (Lokapirnasari et al., 2024). Quail meat has gained a lot of popularity in many nations due to its high protein content and other vital elements. The production of quail has a number of restrictions and difficulties. One of these is vulnerability to bacterially-induced illnesses (El-Ghany, 2019). The prevalence of various bacterial isolates that can be found in quail, one of which is Klebsiella pneumonia (Kahin et al., 2024). Based on research conducted by Hamad et al. (2012) on quail intestinal specimens, the isolated bacteria, one of which is *K. pneumoniae*, was 7.02%.

K. pneumoniae is a Gram-negative, short, straight rod-shaped bacterium, arranged singly or in pairs, measuring approximately 0.5-0.8 µm, non-motile, and non-sporing. The accumulation of extracellular polysaccharides gives Klebsiella colonies a mucoid appearance (Abbas et al., 2024). Host-to-host *K. pneumoniae* infection requires close contact and generally occurs via the fecal-oral route. In the natural environment, the initial mucosal colonization sites tend to be the oropharynx and gastrointestinal tract (Young et al., 2020). This colonization event is generally asymptomatic. Klebsiella colonizes various sites in the body such as the nasal and digestive tracts. This colonization can progress to infection when the host's immune system fails to control the growth of the pathogen, and when the immune system is compromised (Chang et al., 2021). To survive well in the host, *K. pneumoniae* utilizes the help of its virulence factors.

Identified virulence factors of *K. pneumoniae* include capsules, lipopolysaccharides, siderophores, and fimbriae (pili) (Ko, 2017). The most extensively researched fimbriae are types 1 and 3. Mannose-sensitive fimbriae, or type 1 fimbriae, have the ability to bind dissolved mannose as a competitive inhibitor. The primary fimbrial subunit, FimA, a minor apical adhesion protein, and FimH make up type 1 fimbriae (Schembri and Klemm, 2001). FimH contains mannose and encourages adherence to the host surface (Li and Ni, 2023). FimH is an adhesion factor related to biofilm formation. One significant virulence feature of bacteria is their capacity to create biofilms, which are the primary cause of a large number of chronic infections (Sharma et al., 2023).

Some microbes, such as *K. pneumoniae*, have a significant pathogenicity trait: the capacity to create biofilms. Highly organized microbial populations known as biofilms show heightened resistance to host defenses and antimicrobial agents (Uruén et al., 2020). Biofilms shield bacteria from antibiotics and immunological reactions (Guerra et al., 2022). Through the formation of biofilms that increase their persistence on the organism's tissue surfaces, *K. pneumoniae* can shield the germs from harmful host circumstances (such as hypoxia and nutrition deprivation) and antimicrobial medications (Li et al., 2024). Resistance to antimicrobial medicines is 10–1000 times stronger in the biofilm state of *K. pneumoniae* than in the planktonic stage, and biofilms are closely linked to high levels of resistance in clinical isolates of the bacteria (Huang et al., 2022). This can complicate diagnosis and treatment.

The FimH gene was 91% identified from *K. pneumoniae* isolated from anal swabs of laying hens and the environment in chicken farms in Xinjiang, China (Hou et al., 2024). Information regarding the FimH gene in quail has not been widely reported, so the aim of this study was to determine the FimH virulence gene in quail in Indonesia.

ISSN: 2090-6277/2090-6269/

Materials and methods

Ethical approval

Ethical approval for this study was obtained from the Animal Ethics Committee, Faculty of Veterinary Medicine, Wijaya Kusuma University Surabaya, Indonesia (Ethics Number: 170-KKE-2025).

Sampling

The number of samples used was 130 quail cloacal swabs taken from 5 markets in Surabaya City consisting of Turi market, Cemara Pabean market, Bratang market, Kupang market and Benowo market. The collection was carried out using sterile cotton swabs (Onemed, Indonesia) and inserted into the transport media, namely Buffer Pepton Water 1% (Merck 1.07228.0500) and the specimens were stored in a cool box with an ice pack (Permatasari et al., 2022).

Isolation and identification of K. pneumoniae

Samples were cultivated by streaking on Mac Conkey Agar (MCA) and incubated at 37°C for 18-24 hours, then observations were made on the growing colonies. *K. pneumoniae* in MCA has large, pink, regular, round, smooth, raised and slimy colonies. Colonies with these characteristics were identified both microscopically with Gram staining and biochemically using Triple Sugar Iron Agar (TSIA) (HiMedia M021) and iMViC (Sulfide Indole Motility (SIM) (HiMedia M181), Methyl Red-Voges Proskauer test (Himedia GM070), and Simmon's Citrate test (HiMedia M099) (He et al., 2022; Putra et al., 2019).

PCR test of FimH gene

The test was carried out by adding bacterial colony scrapings to 200 ul of TE buffer (10 mM Tris, pH 8, 10 mM EDTA) which had been put into a 1.5 ml tube, then vortexed for 15 seconds. Let stand at room temperature. Centrifugation at 10,000 rpm for 10 minutes. Then mix PCR Master mix (Promega) 12.5 µl, NFW 0.5 µl, Primer forward and Primer reverse (Table 1) 1 µl and DNA template 5 µl in an ependorf tube then amplification was carried out. Primer optimization at the confirmation stage was carried out with an annealing temperature range of 58˚C with a target product weight of 680 bp. The thermal cycler protocol consists of 5 minutes of pre-denaturation at 94°C, 30 seconds of denaturation at 94°C, 30 seconds of annealing at 59°C, and 30 seconds of extension at 72°C. After 35 repetitions of the cycle, a final extension is performed for five minutes at 72°C. PCR products (amplicons) are separated using an electrophoresis machine. Agarose gel electrophoresis is carried out by reacting PCR products in 2% agarose gel, namely 1.2 mg of agarose plus 60 ml of TBE buffer once and homogenized by microwave at 56°C for 2 minutes. After melting, it is allowed to stand until it reaches 50°C and then stained with Sybr Safe, then poured into the gel mold with a comb and left for 30 minutes to solidify. Next, the comb is removed and the agarose gel is ready to use. PCR products of 10 µl and 7 µl of 100 bp DNA ladder (Invitrogen) were poured into agarose gel wells and then run at 150 Volts for 30 minutes (Effendi et al., 2018; Naga et al., 2021).

Table 1. Primers used to detect the fimH gene in *K. pneumoniae* bacteria.

Primer	Sequence 5'-3'	Annealing temperature	Reference
FimH	F: TGCTGCTGGGCTGGTCGATG R: GGGAGGGTGACGGTGACATC	58°C	(Naga *et al*., 2021)

Results

Isolation and identification of Klebsiella pneumoniae

The results of isolation and identification of 130 quail cloacal swab samples from markets in Surabaya City were found to be positive for *K. pneumoniae* by 10% (13/130) (Table 2). Positive samples of *K. pneumoniae* in MCA (Figure 1) showed pink colonies and mucoid colonies. Gram staining of *K. pneumoniae* (Figure 2) showed Gram-negative results, single or paired short rods. Biochemical tests with TSIA and iMViC tests showed that the bacteria ferment lactose and glucose, acidic/acidic TSIA with gas, H2S negative, non-motile, do not produce indole, Citrate positive, MR negative and VP positive (Figure 3).

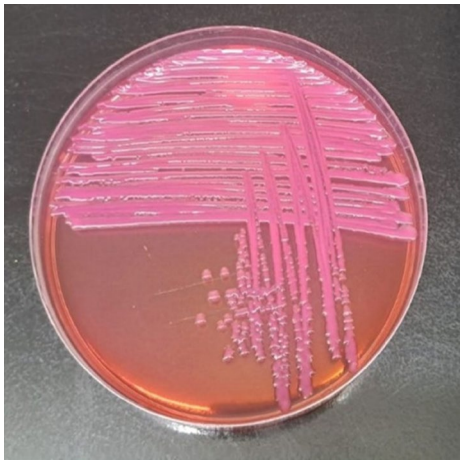

Figure 1. Isolation of *K. pneumoniae* bacterial colonies on Mac Conkey Agar (MCA) media.

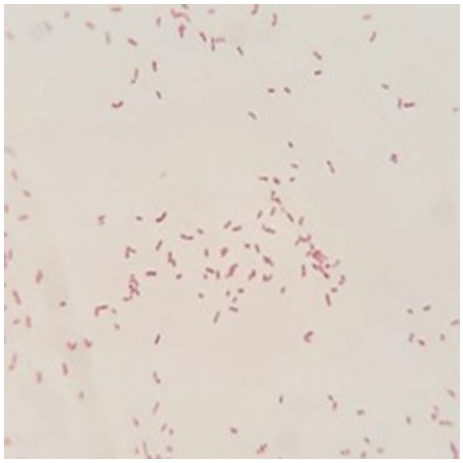

Figure 2. Morphology of *K. pneumoniae* bacteria with Gram staining at 1000x magnification).

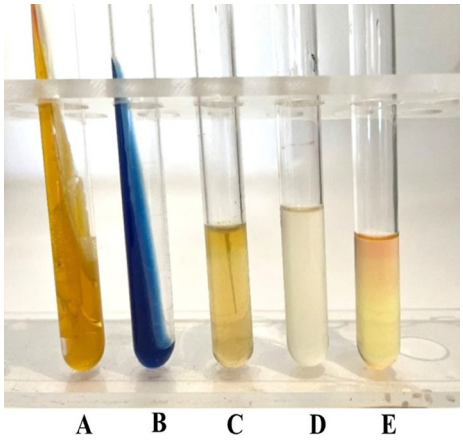

Figure 3. The results of biochemical tests of bacterial isolates identified as bacteria.

Detection of the FimH gene

In this study, 13 *K. pneumoniae* bacteria were found positive from quail cloacal swabs in the Surabaya City market, followed by detection of the FimH virulence gene using the PCR method. Based on the results of this study, 85% (11/13) of *K. pneumoniae* isolates from quail cloacal swabs in the Surabaya City market showed a positive band for the FimH gene at 580 bp (Figure 4).

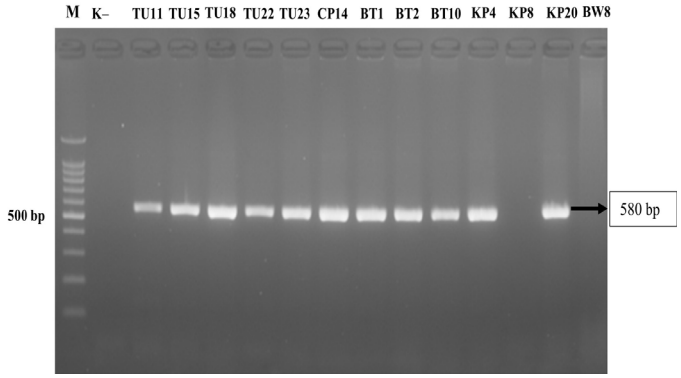

Figure 4. The results of gel electrophoresis products on *K. pneumoniae* bacterial samples detected the presence of the FimH gene.

Table 2. Number of positive samples of *K. pneumoniae* bacteria.

Market of origin	Number of samples	Number of positive samples	Positive sample code
Turi (TU)	26	19% (5/26)	TU 11 TU 15 TU 18 TU 22 TU 23
Cemara Pabean (PB)	26	4% (1/26)	CP 14
Bratang (BP)	26	12 % (3/26)	BT 1 BT 2 BT 10
Kupang (KP)	26	12 % (3/26)	KP 4 KP 8 KP 20
Benowo (BW)	26	4% (1/26)	BW 8

Discussion

In this study, *K. pneumoniae* isolates obtained from quail cloacal swabs in the Surabaya City market showed that 85% (11/13) of the samples were positive for carrying the fimH gene, which was detected through PCR testing in a 580 bp fragment. This finding indicates that most isolates have high virulence potential, because this gene plays an important role in the mechanism of bacterial adhesion to host cells. This result is in line with the study of Alwan et al. (2024) who reported the detection of the FimH gene in 100% of *K. pneumoniae* isolates with an amplicon size of around 550 bp, indicating that the presence of this gene is a common character in many *K. pneumoniae* isolates from various sources, including the environment and animals.

The FimH gene encodes the minor subunit of FimH, an adhesin protein located at the tip of type 1 fimbriae, which plays a role in bacterial attachment to the surface of host epithelial cells. Type 1 and 3 fimbriae are fine filamentous structures that protrude from the surface of bacterial cells and are the primary mediators of initial colonization during infection. According to Liu et al. (2023), type 1 fimbriae are a key pathogenicity factor for *K. pneumoniae*, enabling the bacteria to adhere to the mucosal tissues of the respiratory, intestinal, and urinary tracts. This adhesion process is a crucial initial step in biofilm formation, which then strengthens the bacteria's ability to survive in unfavorable environments, including within the host body and on the surfaces of livestock equipment (Mahmood and Abdullah, 2015).

Structurally, the type 1 fimbriae of *K. pneumoniae* are homologous to the type 1 fimbriae of *Escherichia coli* (Qin et al., 2022). Its genetic complex consists of several genes, including FimA and FimH, where FimA encodes the main structural protein forming the fimbrial rod, while FimH encodes a terminal adhesin that binds to a mannose-sensitive glycoprotein receptor on host cells. Therefore, the interaction between type 1 fimbriae and this receptor is known as "mannose-sensitive binding" (Paczosa and Mecsas, 2016). In addition to fimH, other genes such as mrkA and mrkD also contribute to biofilm formation and adhesion, as reported by Ashwath et al. (2022), who showed that the expression of these three genes is associated with higher levels of biofilm formation and virulence in clinical isolates.

Biofilms are microbial communities arranged in a complex extracellular matrix composed of polysaccharides, proteins, enzymes, and nucleic acids (Zhao et al., 2023). The biofilm structure provides protection against extreme environmental conditions, phagocytosis, and antibiotic exposure (Almatroudi, 2025). In the context of infection, the presence of biofilms is a major cause of chronic infections because it makes it more difficult for bacteria to be eliminated by both the immune system and antimicrobial therapy. According to Mahmood and Abdullah (2015), biofilm formation is a primary defense mechanism for pathogenic bacteria to enhance colonization and persistence in host tissues.

Furthermore, Mirghani et al. (2022) explained that biofilms also play a crucial role in antibiotic resistance mechanisms, as their matrix layer can inhibit antibiotic penetration into the interior of bacterial cells. Bacteria living within biofilms exhibit resistance levels up to 1,000 times higher than planktonic bacteria (Muteeb et al., 2023). This explains why antibiotic therapy often fails to treat infections caused by biofilm-forming bacteria such as *K. pneumoniae*. Furthermore, irrational antibiotic use can accelerate the selection of resistant bacteria, resulting in recurrent infections that are difficult to treat (Khairullah et al., 2019).

These findings indicate that poultry farms and markets, including quail, can be important reservoirs for *K. pneumoniae* bacteria carrying the FimH virulence factor. With their ability to form biofilms and high resistance tendencies, these isolates have the potential to pose a zoonotic risk and spread antimicrobial resistance to humans through the food chain or direct contact. Therefore, the detection and characterization of virulence genes such as FimH in isolates from animals and market environments has great epidemiological significance in efforts to control the spread of pathogenic *K. pneumoniae* in the community.

Conclusion

The results of this study indicate that 85% (11/13) of the FimH gene was detected in *K. pneumoniae* from quail swabs collected at the Surabaya city market. This can be a concern for implementing and improving sanitation hygiene in the livestock sector. Implementing biosafety and biosecurity in cage management to ensure food safety for the community in accordance with the concept of "safe from farm to table" which is the goal of veterinary public health in creating a healthy Indonesian society.

Acknowledgments

Thank you to Prof. Dr. Mustofa Helmi Effendi, drh., DTAPH who has helped finance all of this research.

Conflict of interest

The authors have no conflict of interest to declare.

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